UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/759,315	01/16/2004	Gregory T. Bleck	GALA 08484	9065

72960 7590 07/17/2009

Casimir Jones, S.C.
440 Science Drive
Suite 203
Madison, WI 53711

EXAMINER	
POPA, ILEANA	
ART UNIT	PAPER NUMBER
1633	

MAIL DATE	DELIVERY MODE
07/17/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/759,315	BLECK ET AL.	
	Examiner	Art Unit	
	ILEANA POPA	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02 March 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-10,12,14-18,20-26,28 and 30-41* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-10, 12, 14-18, 20-26, 28, and 30-41* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Claims 11, 13, 19, 27, 29, and 42 have been cancelled. Claim 1 has been amended.

Claims 1-10, 12, 14-18, 20-26, 28, and 30-41 are pending and under examination.

2. The following rejections are withdrawn in response to Applicant's amendments to the claims filed on 03/02/2009 (specifically, none of the cited references teaches a retroviral vector comprising an exogenous promoter internal to the 5' and 3' LTRs):

The rejection of claims 1-10, 12, 14, 18, 20, 21, 28, 30-34, and 41 under 35 U.S.C. 103(a) as being unpatentable over Mathor et al. (Proc. Natl. Acad. Sci. USA,1996, 93: 10371-10376), in view of each Felts et al. (Strategies,1999, 12: 74-77), Wang et al. (Gene Therapy, 2000, 7: 196-200), Zhou et al. (Mol Endocrinol, 1989, 3: 1157-1164, Abstract), and Inaba et al. (J. Surg. Res., 1998, 78: 31-36);

The rejection of claims 1-10, 12, 14-18, 20, 21, 26, 28, 30-34, and 41 under 35 U.S.C. 103(a) as being unpatentable over Mathor et al. taken with each Felts et al., Wang et al., Zhou et al., and Inaba et al., in further view of Burns et al. (Proc. Natl. Acad. Sci. USA, 1993, 90: 8033-803);

The rejection of claims 1-10, 12, 14, 18, 20, 21, 26, 28, 30-38, and 41 under 35 U.S.C. 103(a) as being unpatentable over Mathor et al. taken with each Felts et al.,

Wang et al., Zhou et al., and Inaba et al., in further view of Schroder et al. (Biotech. Bioeng., 1997, 53: 547-559);

The rejection of claims 1-10, 12, 14, 18, 20-24, 26, 28, 30-34, and 39-41 under 35 U.S.C. 103(a) as being unpatentable over Mathor et al. taken with each Felts et al., Wang et al., Zhou et al., and Inaba et al., in further view of both Primus et al. (Cancer Res., 1997, 53: 3355-3361, of record) and Kolb et al. (Hybridoma, 1997, 16: 421-426, Abstract);

The rejection of claims 1-10, 12, 14, 18, 20, 21, 25, 28, 30-34, and 41 under 35 U.S.C. 103(a) as being unpatentable over Mathor et al. taken with each Felts et al., Wang et al., Zhou et al., and Inaba et al, in further view of Naldini et al. (Science, 1996, 272: 263-267).

Response to Arguments

Double Patenting

3. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a

nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

4. Claims 1-10, 12, 14-18, 20-26, 28, and 30-41 remain provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 36-74 and 94-102 of copending Application No. 11/928,464, in view of Schroder et al. (Biotech. Bioeng., 1997, 53: 547-559, of record).

This is a provisional obviousness-type double patenting rejection.

The instant claims are drawn to a method for transducing host cells by providing an immortal host cell and a plurality of retroviral vectors encoding a gene of interest, contacting the host cell at a multiplicity of infection from about 10 to 1000, repeating the above steps a plurality of time, clonally selecting the host cell expressing the gene of interest, and purifying the protein of interest (claims 1-10, 25, 28, 30, and 31). The retroviral vector is pseudotyped and comprises MoMLV elements, an exogenous promoter, a signal sequence, and an amplifiable marker such as DHFR (claims 12, 14-18, 20, 35, and 36) and the vector encodes at least two proteins, such as immunoglobulin heavy and light chains, arranged in a polycistronic sequence (i.e., the retroviral vector comprises IRES) (claims 22-24 and 39). Clonally selected cells are cultured in the presence of a selection agent such as methotrexate and could express 1, 10, or 50 pg per cell per day of the protein of interest (claims 32-34, 37, and 38), and the host cell comprises from 20 to about 100 integrated retroviral vectors (claim 41).

The host cell can be a CHO or a 293 cell (claim 26) and the host cell can be transduced with at least two different vectors encoding different genes of interest (claim 40).

The application claims recite a method of transfecting a host cell and producing a protein of interest by providing a host cell and retroviral vectors comprising an exogenous promoter, a gene encoding for a protein of interest, contacting the host cell with the retroviral vector at a multiplicity of infection of 1000, and culturing the transduced host cell such that the protein encoded by the gene of interest is produced, wherein between 2 and 1000 copies of retroviral vector integrate into the host cell genome; the host cell could be clonally selected and the protein of interest is further isolated (claims 36-43, 49, 51, 54, 56, 58-67, 69, 71-74, and 94-101). The retroviral vector is pseudotyped and comprises MoMLV elements, a signal sequence, an RNA stabilizing element IRES, at least two gene of interest such as the immunoglobulin genes arranged in a polycistronic sequence, the host cell is a CHO cell (i.e., immortal cell), the host cell secretes 1, 10, or 50 pg per cell per day of the protein of interest, and the host cell could comprise a second retroviral vector encoding a second protein of interest (claims 36-38, claims 44-48, 50, 52, 53, 55, 57, 68, 70, and 102). The application claims do not recite DHFR and methotrexate. Schroder et al. teach the amplification of hATIII expression in CHO cells via DHFR-mediated gene amplification in the presence of methotrexate (Abstract, Introduction, Table I). It would have been obvious to one of skill in the art, at the time the invention was made, to include an amplifiable marker, such as DHFR, into the instant vector and select with methotrexate for increased protein production, with a reasonable expectation of success. One of skill

in the art would have been motivated to do so because Schroder et al. teach that increase synthesis of recombinant proteins in animal cells is commonly achieved by using gene amplification. One of skill in the art would have been expected to have a reasonable expectation of success in making and using such a composition because the art teaches that such a composition can be successfully made and used.

Thus, at the time of the invention, one of skill in the art would have considered the instantly pending claims an obvious variation of the application claims when viewed in light of the teachings of Schroder et al.

Applicant asserts that he has filed a terminal disclaimer. However, it is noted that a terminal disclaimer over Application No. 11/928,464 is not on file. For this reason, the rejection is maintained.

New Rejections

Claim Rejections - 35 USC § 103

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. Claims 1-10, 12, 14-18, 20, 21, 28, 30-34, and 41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mathor et al. (Proc. Natl. Acad. Sci. USA,1996, 93: 10371-10376, of record), in view of each Burns et al. (Proc. Natl. Acad. Sci. USA, 1993,

90: 8033-8037, of record), Felts et al. (Strategies,1999, 12: 74-77, of record), Schott et al. (Somatic Cells and molecular Genetics, 1996, 22: 291-309), and Persons et al. (Blood Cells, Molecules, and Diseases, 1998, 24: 167-182).

Mathor et al. teach a retroviral vector encoding human interleukin 6 (hIL-6), wherein the retroviral vector contains MoMLV LTRs, wherein the vector is used to transduce keratinocytes at a MOI of 30, wherein the keratinocytes integrate multiple proviral copies in their genome, and wherein the transduced keratinocytes secrete hIL-6 at a rate of approximately 800 ng per 10^6 cells per day during their lifetime (i.e., the cells secrete more than 1 pg per cell per day); the transduced cells are grown as mass cultures or are cloned by limiting dilution (claims 1, 18, 20, 28, 31, 32) (Abstract, p. 10371, column 2, second paragraph, Material and Methods, p. 10372, columns 1 and 2, p. 10373, column 2). Since hIL-6 is secreted, the retroviral vector must necessarily comprise a segment encoding a secretion signal sequence operably linked to the gene encoding for hIL-6 (claim 21). Mathor et al. teach clonal analysis by Southern blot and by radioimmunoassay, wherein the radioimmunoassay is performed on isolated hIL-6 (claims 1 and 30) (p. 10372, columns 1 and 2, p. 10374, p. 13075, column 1 and Fig. 4, p. 10636, column 1). Mathor et al. teach 11 clones with 1 to 15 proviral integrations, i.e., Mathor et al. teach clonally selecting at least 1 or 10 colonies (claims 35 and 36) (p. 10373, Table 1). Mathor et al. also teach that the retroviral vector is produced from packaging cell lines transfected with an envelope plasmid and a vector plasmid, wherein the packaging cell line expresses gag and pol proteins (claims 12 and 14) (p. 10371, column 2 bridging p. 10372).

Mathor et al. do not teach immortal cells (claim 1), nor do they teach 293-GP cells (claim 15), VSV-G protein (claims 16 and 17). Burns et al. teach producing retroviral vectors pseudotyped with VSV-G, wherein the vectors are produced in 293-G cells and wherein the pseudotyped retroviral vectors are able to mediate stable gene transfer in cell lines such as the BHK cell line (i.e., immortal cells) (Abstract, p. 8033, columns 1 and 2, p. 8035, column 1, second paragraph). Based on these teachings, one of skill in the art would have known that immortal cells could also be used in the method of Mathor et al. and would be motivated to modify the method of Mathor et al. by substituting their secondary cells with immortal cells to achieve the predictable result of obtaining consistent production of desired proteins for unlimited time. Furthermore, it would have been obvious to one of skill in the art, at the time the invention was made, to modify the method of Mathor et al. by using the pseudotyped retrovirus of Burns et al., with a reasonable expectation of success. The motivation to do so is provided by Burns et al., who teach that such a virus has an expanded host range (Abstract, p. 8033, column 2). One of skill in the art would have been expected to have a reasonable expectation of success in making and using such a composition because the art teaches that such a composition can be successfully made and used.

Mathor et al. and Burns et al. do not specifically teach serial transduction to a obtain cell comprising in its genome from 20 to about 100 integrated vectors (claims 1-10 and 41). However, Mathor et al. do teach that protein expression is directly proportional to the integration events (i.e., copy number) (p. 10376, column 1). Additionally, the prior art as a whole teaches that there is a positive correlation between

the MOI and integration events. For example, Felts et al. teach that the advantage of retroviral vectors is that the copy number of integrated provirus can easily be controlled by varying the multiplicity of infection (MOI) (p. 74). Schott et al. teach serially transducing cells with a retroviral vector carrying an internal promoter driving the expression of a gene of interest, wherein higher MOI result in higher integration events and wherein the expression and stability of the gene of interest directly correlates with the number of integrated retroviral vectors (Abstract, p. 292, column 2, p. 294, column 2, second paragraph, p. 295, column 1, p. 302, column 2, first full paragraph, p. 303, column 2 and Fig. 9, p. 308, column 1). Persons et al. teach that repeatedly transducing cells with retroviral vectors at a MOI of 1,000 results in cells comprising 20 copies of integrated retroviral vector (Abstract, paragraph bridging p. 168 and 169, p. 171, column 2, last paragraph, p. 172, column 2, last paragraph, p. 173, column 2, p. 174, column 2, p. 177, column 2, p. 179, column 1, first full paragraph). Based on these teachings, one of skill in the art would have known that serially transducing cells with high MOI would result in increased proviral integration events. It would have been obvious to one of skill in the art, at the time the invention was made, to modify the method of Mathor et al. and Burns et al. by serially transducing their cells with high MOIs (such as MOIs of 1,000) to achieve the claimed ranges of integration events, with a reasonable expectation of success. The motivation to do so is provided by Mathor et al., who teach the possibility of specifying the level of transgene expression by controlling the integration events (Abstract, p. 10376, column 1). One of ordinary skill in the art would have been expected to have a reasonable expectation of success in doing

so because the art teaches that the level of retroviral vector integration events can be easily controlled by manipulating the MOI. With respect to the limitation of an internal promoter (claim 1), using such was routine in the prior art, as taught by Schott et al. (p. 292, column 2). It would have been obvious to one of skill in the art, at the time the invention was made, to modify the method of Mathor et al. and Burns et al. by further including an internal promoter in their vector to achieve the predictable result of expressing hIL-6 in their cells. With respect to the limitations of one cell secreting more than 10 or 50 pg protein per day (claims 33 and 34), one of skill in the art would have had known to obtain the desired amounts of synthesized proteins by controlling the number of integration events.

Thus, the claimed invention was *prima facie* obvious at the time the invention was made.

Applicant's arguments are answered below to the extent that they pertain to the instant rejection.

Applicant argues that Dr. Bleck specifically addressed the data contained in Mathor in paragraph 11 of his declaration: The data [in Mathor] shows that at 8 integrations, 1140 ng/106 cells/day of protein is produced, and that when 15 integrations were obtained, the protein production decreased to 1014 ng/106 cells/day or protein produced. This indicates that protein production had reached a plateau and that further introduction of retroviral vectors did no good or decreased protein production. Thus, one of skill in the art would conclude from the data additional

integration past 8 integrations were not needed or not desirable. At page 19 of the Office Action, the Examiner responds to Second Bleck Declaration and argues that Table 1 of Mathor et al. shows the result obtained with only one keratinocyte clone for each of the 8 and 15 integration events. Based on this observation, the Examiner then states: "Such results cannot be extrapolated to all clones. The art teaches that retroviral insertion is random and that expression level is dependent on the insertion sites (see Mathor et al., p. 10376, column 1; Liu et al., Anal Biochem, 2000, 280:20-28, Abstract, p. 21, column1; Stamps et al., Int J. Cancer, 1994, 57:865-874, Abstract, p. 868, column 1, p. 869, Fig. 2). Based on these references, one of skill in the art would expect clones with the same number of copies to have different expression levels, and therefore, would know to select multiple clones and look for the high expressing ones". Applicant submits that this is sheer speculation by the Examiner and that it is the Examiner that is making an unsupported extrapolation of the data. There is no other data except for the clones identified in Table 1 of Mathor. It is this data that is addressed by Dr. Bleck. There is no basis in this data for the Examiner to conclude that a person of skill in the art would be motivated to make cells lines containing more than 20 integrated vectors and select for high expressing clones. In effect, the Examiner ignores the actual data and proceeds to extrapolate the data to support an argument that a person of skill in the art would be motivated to make cell containing 20 integrated retroviral vectors when the data set provides no evidence to support that conclusion. As Dr. Bleck states in his Second Declaration, at most, a person of ordinary skill in the art, considering Mathor, would conclude that protein expression is correlated to integration number only through

about 8 integration events. The Examiner has provided no evidence from which it can be concluded that a person of skill in the art would extrapolate the actual Mathor data to conclude that making cell lines with genomes containing greater than 20 integration events were desired or feasible. Indeed, the evidence, i.e., data, in Mathor argues otherwise as established in the Second Bleck Declaration.

Applicant asserts that Zielske clearly teaches that use of internal promoters such as CMV results in a plateau of expression at 4 copies per cell, and therefore, the claimed invention where multiple copies of the retroviral vector are introduced via multiple transductions and wherein the vectors comprise an internal promoter driving expression of a desired gene is unexpected in view of the art.

Applicant argues that the Examiner has failed to consider the prior art as whole. When viewed as a whole, as required by the law, the prior art establishes that it was not predictable that multiple transductions at high MOI could be used to make viable cell lines containing more than 20 integrated vectors and expressing a protein of interest could be obtained. The results obtained by Applicant simply were not predictable and an obviousness rejection over the cited references is not proper.

There are multiple examples of the fact that the Examiner has chosen to ignore the inconsistent teachings of the prior art and thus has failed to consider the teachings of the prior art as a whole.

As one example, the Examiner states "Applicant argues that malignant transformation of cells in vitro is almost certain to affect the production of the desired protein. Again, this is an assertion not supported by evidence." Office Action, p. 17-18.

This statement was made in relation to the discussion of Coffin et al. in the Second Bleck Declaration. The Examiner has made several errors with respect to the Second Bleck Declaration. First, this passage by the Examiner mischaracterizes the actual evidence in Second Bleck Declaration. The Second Bleck Declaration is not limited to "malignant transformation." In contrast, Paragraph 5 of the Second Bleck Declaration establishes that: "Coffin et al. confirms the teaching of Arai et al. that the incorrect use of retroviral vectors can lead to insertional mutagenesis. Furthermore, the Examiner's assumption that malignant transformation or other mutagenesis would not impede an immortalized mammalian cell from producing a protein of interest has no scientific basis. In fact, if an immortalized mammalian cell is mutagenized or transformed in some way by the vector, it is almost certain that production of the desired protein would be affected. The recombinant protein production industry relies on the use of standardized immortalized mammalian cells whose growth is predictable. Cells with additional mutations would be highly undesirable. Thus, the Bleck Declaration established that cells containing additional mutation would be highly undesirable to the recombinant protein production industry. The Examiner has failed to address this evidence.

Second, the Bleck Declaration is evidence of what one of skill in the art would conclude based on Arai and Coffin (as well as the other references addressed) and cannot be summarily dismissed by the Examiner. Thus, contrary to the Examiner's assertion, Applicants have submitted evidence to support their arguments. The Second Bleck Declaration provides evidence concerning the prior art that the Examiner has

failed to consider or address. Thus, the Examiner has not considered the prior art as a whole.

As another example, the Second Bleck Declaration establishes that Walker et al. teaches that retroviruses and repeated genes are often silenced or suppressed by mammalian cells. Because of viral interference and gene silencing or suppression, a person of ordinary skill in the art would be discouraged from using sequential transductions to increase viral copy number and would be discouraged from attempting to create immortalized mammalian cell lines with the claimed number of insertions. Applicant submits that the Examiner has not considered the entire reference. Walker does teach, as the Examiner notes, that, transduction of the cell with one viral vector type interferes with the cell being transduced by another cell type. However, Walker goes on to teach at p. 1137, column 2, that the prior art recognized that "viral interference resulted when susceptible cells were rendered resistant to specific retroviral infections by preinfection with a virus bearing the same glycoprotein specificity (Weiss, 1984) ....This is thought to occur because the leukemia virus particles bear envelope proteins similar to sarcoma virus envelope glycoproteins, which competitively block the cell receptors and prevent binding by RSV viral particles." After ruling out this type of interference in their study, Walker et al. concluded that "The data suggest that the interference we observed with sequential retroviral transductions may occur at the level of retroviral DNA integration and or expression. Possibly retroviral DNA integration is not as random as currently thought." Walker et al., p. 1137, column 2. Thus, what Walker et al. were testing was whether the same viral interference observed when

vectors with similar glycoprotein specificities are used in a transduction protocol would be observed when vectors with different glycoprotein specificities were used. They not only found that viral interference existed, but that quite likely the interference existed at the level of integration and or expression. Thus, a person of skill in the art would recognize that these findings are applicable to situations where vectors with the same glycoprotein specificity are used (which applies to use of the same vector) as well as to situations where different vectors are used.

As still another example, the Examiner argues that: "With respect to Bestor, the reference is related to silencing *in vivo* and not cells *in vitro*. For example, Bestor teaches that, while fibroblasts transduced with retroviral vectors stably expressed adenosine deaminase in vitro, transplantation of these cells into mice resulted in decreased adenosine deaminase expression (p. 409, column 2, last paragraph). This is only one example from Bestor. Bestor generally teaches that:

- In mammals, the insertion of retroviral DNA or the incorporation of repeat arrays can trigger transcriptional silencing of the inserted sequences, usually via mechanisms that involve methylation of DNA within regulatory regions. P. 409, column 1.
- I will argue here that gene silencing mechanisms are diverse and efficient and are likely to represent a barrier to many forms of gene therapy.
- The existence of gene silencing, the recognition and inactivation of alien genes by target cells (reviewed in ref. 1), has only recently been recognized as an additional challenge to gene therapy.

- Many cases are known in which a transferred gene undergoes a brief period of expression followed by a decline to undetectable levels without the loss of the expression construct.
- Repeat-induced gene silencing has clear relevance for any gene therapy approach that is likely to lead to the insertion of multicopy arrays. P. 410, column 1.
- Gene silencing can also occur at the RNA level even while transcription proceeds at high rates. P. 410, column 2.
- It should be noted that the mechanisms involved in virtually every form of gene silencing remain to be discovered, and research in this area is likely to accelerate in the near future. P. 411, column 1.

Applicants further note that the example cited by the Examiner is derived from Palmer et al., Proc. Nat'l. Acad. Sci. USA 88:1330-34 (1991). Applicant has attached this reference. Applicant notes that Palmer et al. do not address copy number of the retroviral vector and thus do not address the statements in Bestor that relate to silencing of multiple copies of retroviral vectors, or just the introduction of multiple copies of the same gene, regardless of vector. A person of skill in the art would recognize that those statements are far more applicable to the state of the prior art on introduction of multiple genes into a host cells as is presently claimed. The importance of these statements is clear: there was a great deal of concern in the prior art that gene silencing would limit the effectiveness of the introduction of multiple copies of a retroviral vector, or indeed, any vector into a target cell. Applicants note that this is consistent with Mathor et al., which shows that expression decreased in cells with 15 copies of a vector as compared

to cells with 8 copies of a vector. Mathor shows that increasing copy number past a certain point resulted in a decrease in expression.

As a final example, the Examiner continues to rely on Kustikova et al. and Zielske et al. references (which are not prior art) to rebut Applicant's arguments based on Arai and Coffin. Applicant notes the content of the prior art must be analyzed as it existed "at the time the invention was made" to avoid impermissible hindsight. MPEP 2141.01 III. It simply is not proper to use a reference that is not prior art to rebut Applicants arguments regarding the scope and content of the prior art at the time the invention as made. Applicants arguments based on Arai and Coffin relate to the scope and content of the prior art as of the priority date of the application. It is contrary to both the MPEP and the established precedent of the Supreme Court to use references published after the invention was made to rebut such arguments. Effectively, the Examiner has made a post-filing date reference part of the scope and content of the prior art.

Accordingly, Applicant argues, since the Examiner has not established a *prima facie* case of obviousness, the rejection should be withdrawn.

Applicant's arguments are acknowledged, however, the rejection is maintained for the following reasons:

Applicant argues that the Second Bleck Declaration establishes that one of skill in the art would not extrapolate the data of Mathor et al. to conclude that making cell lines with genomes comprising greater than 20 integration events was desired or

feasible. The Second Bleck Declaration was previously presented and addressed. Furthermore, apart from an argument, Applicant and his Declaration did not provide any evidence indicating that obtaining host cells with genomes comprising greater than 20 integrated viral vectors was not feasible before the instant invention was made. It is noted that Table 1 (to which Applicant refers to) shows the result obtained with only one keratinocyte clone for each 8 and 15 integration events. Such results cannot be extrapolated to all clones. The Examiner provided evidence to support this statement and it is not clear why Applicant argues that such a statement is sheer speculation and an unsupported extrapolation of the data. It is well established in the art that retroviral insertion is random and that expression level is dependent on the insertion sites (see Mathor et al., of record, p. 10376, column 1; Liu et al., of record, Anal Biochem, 2000, 280: 20-28, Abstract, p. 21, column 1; Stamps et al., of record, Int J Cancer, 1994, 57: 865-874, Abstract, p. 868, column 1, p. 869, Fig. 2). It is noted that Applicant did not indicate why the teachings of Liu et al. and Stamps et al. do not support the Examiner's statement. Applicant points to Table 1 in Mathor et al. for support. In fact, Table 1 in Mathor et al. clearly demonstrates that results obtained with one clone cannot be extrapolated to other clones. Specifically, Table 1 demonstrates that three different clones each comprising 3 copies of integrated vectors secrete different amounts of hIl-6 (180, 150, and 450 ng/10^6 cells/day, respectively); two different clones comprising 4 copies of integrated vectors also secrete different amounts of hIl-6 (522 and 449 ng/10^6 cells/day, respectively). Therefore, the Examiner's statement is supported by the art, including the data in Table 1 of Mathor et al. Based on these teachings, one of skill in

the art would expect clones with the same number of copies to have different expression levels, and therefore, would know to select multiple clones and look for the high expressing ones. In fact, as indicated in the rejection above, the prior art does teach obtaining stable host cells comprising 20 integrated copies of retroviral vectors. The prior art also teaches that the number of integrating events can be controlled by varying the MOI and that gene expression directly correlates with the number of integrated copies (see the rejection above). Based on the teachings in the art, one of skill in the art would have known how to introduce 20 or more copies of retroviral vector into a host cell genome. Therefore, the Examiner did not ignore the actual data and the statement that it would have been obvious and within the capabilities of one of skill in the art to obtain host cells with 20 or more integrated copies is not sheer speculation or unsupported extrapolation of the data by the Examiner.

Applicant argues that, since Zielske teaches that use of internal promoters such as CMV results in a plateau of expression at 4 copies per cell, is unexpected in view of the art. This is not found persuasive. Zielske teaches that the limit in transgene expression is related to the particular vector/promoter/transgene/host cell system used in his experiments (p. 926, paragraph bridging columns 1 and 2, p. 929, column 1, third paragraph). In support of these teachings, Schott et al. disclose that protein expression from retroviral vectors comprising an internal CMV promoter does not reach a plateau when increasing the copy number above 4. Moreover, the claims are not limited to the CMV promoter; they recite any internal promoter. The prior art teaches successful

expression from internal promoters in general, including CMV, and therefore the claimed invention is not unexpected.

According to Applicant, the Bleck Declaration establishes that it is almost certain that additional mutations would affect the production of the desired protein and that the Examiner has failed to address this evidence. This is incorrect, as this argument is not new and was previously addressed. It is noted that, apart from an assumption made by Applicant, the declaration does not provide any evidence establishing that protein production is affected by insertional mutagenesis. In fact, both the art and the instant specification do not support such as assumption, as they provide evidence that introducing high copy numbers (i.e., increasing the risk of insertional mutagenesis) does not affect protein production.

Applicant argues that the Bleck Declaration is evidence of what one of skill in the art would conclude based on Arai and Coffin and that the Examiner has failed to consider or address such. This is again incorrect, as Arai and Coffin were previously addressed. Moreover, the Declaration only provides Applicant's opinion and no evidence.

Applicant argues that the teachings of Walker et al. indicate that interference applies to the use of the same vectors as well as to situations when different vectors are used. Such is not true. The teachings of Walker et al. only pertain to using two different retroviral vectors. There is not teaching in Walker et al. (or the art) that interference occurs when using only one vector type. In fact, the prior art teaches integration of 20 copies per cell genome when using one retroviral vector type (see above), thus

providing evidence that interference does not occur when the same viral vectors are used for transduction. Therefore, by reading Walker et al. and taking in account the art as a whole, one of skill in the art would not consider that interference occurs when only one retroviral vector type is used.

With respect to Bestor, the whole reference relates to silencing *in vivo*. There is no teaching in Bestor related to silencing *in vitro*. The only paragraph related to the *in vitro* situation in Bestor discloses that, while fibroblasts transduced with retroviral vectors stably expressed adenosine deaminase *in vitro*, their transplantation into mice resulted in decreased adenosine deaminase expression. Clearly, by reading Bestor, one of skill in the art would understand that his teaching are only referring to silencing *in vivo* and would not extrapolate his teachings to the *in vitro* situation. Moreover, the prior art teaches stable expression of transgenes *in vitro* (i.e., no silencing) when 20 copies of viral vectors are integrated into the genome (see above). For these reasons, Applicant's argument that Bestor's teachings apply to transduction *in vitro* is not found persuasive. For the same reasons, Applicant's argument that Palmer et al. do not address copy numbers is not found persuasive.

Applicant's argument that the Examiner improperly relies on post-filing art is not new and has been answered before. Applicant argues that it is contrary to both the MPEP and the established precedent of the Supreme Court to use references published after the invention was made to rebut Applicant's arguments. In response to this argument it is noted that there is nothing in MPEP indicating that post-filing art cannot be used to provide evidence of what one of skill in the art would have known before an

invention was made. There is nothing in MPEP indicating that post-filing art cannot be used to rebut Applicant's arguments that, based on the teachings in the prior art, one of skill in the art would have been discouraged to arrive at the claimed invention. Similarly, Applicant did not provide any evidence of any established precedent of the Supreme Court. The post-filing references provide evidence that the prior art did not teach away from the claimed invention. Regardless, this argument is irrelevant because, since the art at issue was not used in the rejection, such argument does not address the instant rejection.

For the reasons set forth above, Applicant's argument that the Examiner did not consider the art as a whole is not found persuasive. In conclusion, the prior art teaches how to obtain higher integration events, and therefore, it would have been within the knowledge and capability of one of skill in the art to achieve such. Therefore, the rejection is maintained.

7. Claims 1-10, 12, 14-18, 20, 21, 26, 28, 30-38, and 41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mathor et al. taken with each Burns et al., Felts et al., Schott et al., and Persons et al., in further view of Schroder et al. (Biotech. Bioeng., 1997, 53: 547-559, of record).

The teachings of Mathor et al., Burns et al., Felts et al., Schott et al., and Persons et al. are applied as above for claims 1-10, 12, 14-18, 20, 21, 28, 30-34, and 41. Mathor et al., Burns et al., Felts et al., Schott et al., and Persons et al. do not teach DHFR and culturing the transduced cells in the presence of methotrexate (claims 35-

38), nor do they teach Chinese hamster ovary (CHO) cells (claim 26). Schroder et al. teach the amplification of hATIII expression in CHO cells via DHFR-mediated gene amplification in the presence of methotrexate (Abstract, Introduction, Table I). It would have been obvious to one of skill in the art, at the time the invention was made, to include an amplifiable marker, such as DHFR, into the vector of Mathor et al., Burns et al., Felts et al., Schott et al., and Persons et al. for increasing protein production and to use the modified vector for the transduction of CHO cells, with a reasonable expectation of success. One of skill in the art would have been motivated to do so because Schroder et al. teach that increase synthesis of recombinant proteins in animal cells is commonly achieved by using gene amplification. One of skill in the art would have been motivated to use CHO cells because they are known to be an excellent model cell line for the production of high levels of proteins of interest. One of skill in the art would have been expected to have a reasonable expectation of success in making and using such a composition because the art teaches that such a composition can be successfully made and used. Thus, the claimed invention was *prima facie* obvious at the time the invention was made.

Applicant argues that Schroder et al. do not cure the deficiencies noted above. Applicant's argument is acknowledged, however, the rejection is maintained for the reasons above.

8. Claims 1-10, 12, 14-18, 20-24, 26, 28, 30-34, and 39-41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mathor et al. taken with each Burns et al., Felts et al., Schott et al., and Persons et al., in further view of both Primus et al. (Cancer Res., 1997, 53: 3355-3361, of record) and Kolb et al. (Hybridoma, 1997, 16: 421-426, Abstract, of record).

The teachings of Mathor et al., Burns et al., Felts et al., Schott et al., and Persons et al. are applied as above for claims 1-10, 12, 14-18, 20, 21, 28, 30-34, and 41. Mathor et al., Burns et al., Felts et al., Schott et al., and Persons et al. do not teach at least two different vectors encoding different genes of interest (claim 40). Primus et al. teach a method of expressing a monoclonal IgG2a antibody into a tumor cell, wherein the tumor cell is transduced with two different vectors, one encoding the heavy and the other encoding the light chain (claim 40), and wherein the transduced tumor cell produces self-reactive antibodies (Abstract, p. 3355, column 1, p. 3356, column 1, first full paragraph, p. 3360, column 2). It would have been obvious to one of skill in the art, at the time the invention was made, to use the method of Mathor et al., Burns et al., Felts et al., Schott et al., and Persons et al. to express antibodies into a cancer cell, as taught by Primus et al., with a reasonable expectation of success. The motivation to do so is provided by Primus et al., who teach that antibody gene transfer into autologous tumor cells offer a new and alternative application in the use of antibodies for the immune therapy of cancer. One of skill in the art would have been expected to have a reasonable expectation of success in making such a composition because the art teaches that such a composition can be successfully obtained.

Mathor et al., Burns et al., Felts et al., Schott et al., Persons et al., and Primus et al. do not teach the two genes of interest being arranged in a polycistronic sequence, wherein the genes of interest are the immunoglobulin heavy and light chains (claims 22-24 and 39). Kolb et al. teach concurrent synthesis of both heavy and light chains of the monoclonal antibody A1 by using a bicistronic expression cassette comprising an internal ribosomal entry site (IRES) (Abstract). It would have been obvious to one of skill in the art, at the time the invention was made, to modify the method of Mathor et al., Burns et al., Felts et al., Schott et al., Persons et al., and Primus et al. by using the expression cassette of Kolb et al. for the production of monoclonal antibodies of interest, with a reasonable expectation of success. The motivation to do so is provided by Kolb et al., who teach that their method allows for the rapid isolation of cell clones expressing high levels of recombinant antibody. One of skill in the art would have been expected to have a reasonable expectation of success in making such a composition because the art teaches that such a composition can be successfully obtained.

Thus, the claimed invention was *prima facie* obvious at the time the invention was made.

Applicant argues that neither Primus et al. nor Kolb et al. cure the deficiencies noted above. Applicant's argument is acknowledged, however, the rejection is maintained for the reasons above.

9. Claims 1-10, 12, 14-18, 20, 21, 25, 28, 30-34, and 41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mathor et al. taken with each Burns et al., Felts et al., Schott et al., and Persons et al., in further view of Naldini et al. (Science, 1996, 272: 263-267, of record).

The teachings of Mathor et al., Burns et al., Felts et al., Schott et al., and Persons et al. are applied as above for claims 1-10, 12, 14-18, 20, 21, 28, 30-34, and 41. Mathor et al., Burns et al., Felts et al., Schott et al., and Persons et al. do not teach a lentiviral vector (claim 25). Naldini et al. teach lentiviral vector for the stable transduction of non-dividing cells (Abstract, p. 263, column 1). It would have been obvious to one of skill in the art, at the time the invention was made, to modify the method of Mathor et al., Burns et al., Felts et al., Schott et al., and Persons et al. by using the lentiviral vector of Naldini et al., with a reasonable expectation of success. The motivation to do so is provided by Naldini et al., who teach that their vector can be used for the transduction of non-proliferating cells such as hepatocytes, myofibers, hematopoietic stem cells, and neurons. One of skill in the art would have been expected to have a reasonable expectation of success in using such a composition because the art teaches that such a composition can be successfully used.

Thus, the claimed invention was *prima facie* obvious at the time the invention was made.

Applicant argues that Naldini et al. do not cure the deficiencies noted above. Applicant's argument is acknowledged, however, the rejection is maintained for the reasons above.

Conclusion

10. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ILEANA POPA whose telephone number is (571)272-5546. The examiner can normally be reached on 9:00 am-5:30 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached on 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Ileana Popa/
Primary Examiner, Art Unit 1633